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Protective Effect of Diallyl Disulfide on Ethacrynic Acid-Induced Toxicity in Mice

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Abstract□The present work was undertaken to investigate the effect of diallyl disulfide on ethacrynic acid toxicity. Ethacrynic acid-induced mortality and formation of lipid peroxide were inhibited by diallyl disulfide. Furthermore, decreasing effect of glutathione S-transferase and glutathione level in the liver by ethacrynic acid were reduced by diallyl disulfide. These results suggested that the inducing effect of diallyl disulfide on the ethacrynic acid metabolizing enzyme, glutathione S-transferase, is believed to be a possible detoxication mechanism for the ethacrynic acid toxicity in mice.

Keywords□Diallyl Disulfide, Ethacrynic acid, Glutathione S-transferase, Lipid Peroxidation

We previously reported that the treatment of experimental animal with diallyl disulfide, which is one of the breakdown product[1] of allicin, caused a significant increase of hepatic glutathione S-transferase activity[2]. Such a marked increment of glutathione S-transferase activity may prevent the cell damage due to electrophilic compounds and several organic hydroperoxides[3-7].

Meanwhile, ethacrynic acid which is potent and very effective diuretic contains an α, β-unsaturated ketone moiety, which confers on it a high degree of reactivity toward sulfhydryl group[8]. It is well known that ethacrynic acid is metabolized by glutathione S-transferase *in vivo*[9,10].

Therefore, the present study was designed to investigate the protective effect of diallyl disulfide on ethacrynic acid-inducd toxicity in mice.

EXPERIMENTAL METHODS

Chemicals

Bovine serum albumin and 1-chloro-2,4-dinitrobenzene were purchased from Sigma chemical Co., 5,5,'-dithiobis (2-nitrobenzoic acid) and 2-thiobarbituric acid sodium salt from Nakarai chemical Co., diallyl disulfide from Tokyo Kasei chemical Co., and reduced glutathione from Fluka A.G. Ethacrynic acid was gift from Dr. J.R. Chung. All other chemicals were of reagent grade.

Animal Treatment

ICR-male mice weighing 25 g were used for all studies. Mice were given diallyl disulfide solution (20 mg/kg) intraperitoneally once daily for 5 days. Diallyl disulfide was diluted by the addition of olive oil. Control mice were received olive oil intraperitoneally. All experimental animals were allowed free access to food and water but deprived of food for 16 hr prior to sacrifice.

Acute toxicity

Mice were treated with ethacrynic acid (200 mg/kg) intraperitoneallyl 8 hr after the last dose of diallyl disulfide. Ethacrynic acid was suspended in olive oil. Mice were observed any death within 16 hr.

Preparation of cytosolic fraction

The animals were killed by exsanguination from inferior vena cava. The liver was exhaustively perfused with cold 0.15 M sodium chloride solution through the portal vein until it was uniformly pale and quickly removed. The liver was homogenized in cold 0.25 M sucrose solution by three passes of a motor-driven teflon pastle in a glass homogenizing vessel. The homogenate (20% w/v) was centrifugated at 105,000 *g* for 1 hr and supernatant fraction was used as the cytosolic fraction.

Assay methods

Glutathione S-transferase activity was measured by the amount of thioether formed according to

Habig, *et al.*[4] with 1-cgloro-2,4-dinitpobenzene and glutathione as substrates. Enzyme unit defined as formed thioether n mole per mg protein per min at 25 °C.

Protein concentration was determined by the method of Lowry *et al.*[11] using bovine serum albumin as a standard.

Content of reduced glutathione in liver tissue was estimated by the method of Ellman[12]. Glutathione level was expressed as μmole per g of tissue.

Lipid peroxidation of liver tissue was followed by measuring malondialdehyde with thiobarbituric acid method of Bidlack and Tappel[13]. The concentration of malondialdehyde was expressed as nmole per g of tissue using the molar extinction coefficient of $1.52 \times 10^5\ M^{-1}cm^{-1}$ [14].

Student's t-test was used to estabilish significant differenes in mean values between the control and treated groups.

Table I. Effect of diallyl disulfide on the mortality in ethacrynic acid-treated mice

Treatment	Mortality
Control	70%
Diallyl Disulfide	10%

Mice were given diallyl disulfide (20 mg/kg) intraperitoneally once daily for 5 days. Ethacrynic acid (200 mg/kg) was injected intraperitoneally 8 hr after the last dose of diallyl disulfide.
20 animals were used in each group.

Table II. Effect of diallyl disulfide on the formation of lipid peroxide in ethacrynic acid-treated mouse liver

Treatment	Malondialdehyde (n moles/g of tissue)
Control	15.9 ± 2.9
Control + Ethacrynic acid	58.3 ± 9.4**^{a}
Diallyl disulfide	14.2 ± 3.7
Diallyl disulfide + Ethacrynic acid	34.1 ± 4.4*^{b}

Mice were given diallyl disulfide (20 mg/kg) intraperitoneally once daily for 5 days. Control group were injected with olive oil. Ethacrynic acid (200 mg/kg) was given intraperitoneally 8 hr after the last dose of diallyl disulfide. The assay procedure was described in the text. Values are mean ± S.E of 5 animals.
a; significantly different from the control group, b; significantly different from ethacrynic acid-treated control group.
*; $p<0.05$, **; $p<0.01$

Table III. Effect of diallyl disulfide on the hepatic glutathione level in ethacrynic acid-treated mice

Treatment	Glutathione (μmoles/g of tissue)
Control	5.58 + 0.37
Control + Ethacrynic acid	2.24 ± 0.27***^{a}
Diallyl disulfide	6.69 ± 0.36*^{a}
Diallyl disulfide+ Ethacrynic acid	3.54 ± 0.23**^{b}

The assay procedure was described in the text. The other conditions are the same as described in Table II.
*; $p<0.05$, **; $p<0.01$, ***; $p<0.001$

RESULTS

Effect of diallyl disulfide on acute ethacrynic acid toxicity

The influence of diallyl disulfide on acute ethacrynic acid toxicity in mice is shown in Table I. On pretreatment of diallyl disulfide, the mortality caused by ethacrynic acid decreased greatly.

Effect of diallyl disulfide on the content of hepatic lipid peroxide in ethacrynic acid-intoxicated mice

Table II shows the effect of diallyl disulfide administration on the formation of lipid peroxide in ethacrynic acid-intoxicated mice. Ethacrynic acid administration produced a significant increase in the hepatic lipid peroxide, but the increment of lipid peroxide was remarkably decreased by the pretreatment of diallyl disulfide for 5 days

Effect of diallyl disulfide on the hepatic glutathione level in ethacrynic acid-intoxicated mice

Table III shows the effect of diallyl disulfide on the reduced glutathione content in the liver of mice. By the treatment of diallyl disulfide 5 days, the level of glutathione was increased about 1.2 fold as compared with the control group. When ethacrynic acid was injected to the control group mice, glutathione content was significantly decreased. But decrease in glutathione content in diallyl disulfide pretreated group was lesser than that in control group given ethacrynic acid alone.

Effect of diallyl disulfide on the cytosolic glutathione S-transferase activity in ethacrynic acid intoxicated mouse liver

As shown in Table IV, cytosolic glutathione S-transferase activity in liver was significantly enhanced by the treatment of diallyl disulfide for 5 days. Meanwhile, when ethacrynic acid was injected to control mice, the enzyme activity strikingly decreased. But, in diallyl disulfide pretreated group, the decreasing effect on enzyme activity was reduced.

Table IV. Effect of diallyl disulfide on the hepatic glutathione S-transferase activity in ethacrynic acid-treated mice

Treatment	Specific activity (n moles/mg protein/min)
Control	1140.8 ± 58.3
Control + Ethacrynic acid	714.9 ± 80.7**[a]
Diallyl disulfide	1433.1 ± 75.3*[a]
Diallyl disulfide + Ethacrynic acid	1139.6 ± 51.3**[b]

The assay procedure described in the text. Tha other conditions are the same as described in Table II.
*; p 0.05, **;p 0.01

DISCUSSION

It is also known that garlic has various pharmacological effect[15,16]. Recent studies have shown that garlic components regulate many metabolic diseases, such as atherosclerosis[17,18], diabetes[19,20] and gout[21,22] in experimental animals.

In this report, we have examined the defence mechanism of diallyl disulfide on ethacrynic acid-toxicity in experimental model. Ethacrynic acid is a high ceiling diuretic. However, when ethacrynic acid was given acutely or chronically, it induced many untoward effects involving liver, renal and herat failure[23-25]. It is generally accepted that ethacrynic acid is metabolized by glutathione S-transferase, phase II enzyme[4,9,10].

It was observed that diallyl disulfide reduced the ethacrynic acid-induced acute toxicity in mice. Furthermore, the formation of lipid peroxide by ethacrynic acid was diminished by the pretreatment of diallyl disulfide for 5 days. It is widely accepted that biological membrane damage was represented by lipid peroxide content[26]. This result indicated that ethacrynic acid-induced membrane damage may be prevented by diallyl disulfide.

Decreasing effect of glutathione level and glutathione S-transferase activity in the liver by ethacrynic acid-intoxicated mice were inhibited by diallyl disulfide pretreatment. Glutathione S-transferase is regarded as the detoxifying enzyme which catalyzes the first step in mercapturic acid formation[3-5].

Therefore, these results strongly suggested that diallyl disulfide may prevent the tissue damage due to electrophilic compound such as ethacrynic acid by increment of hepatic glutathione S-transferase.

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LITERATURE CITED

1. Wills, E.D.: Enzyme inhibition by allicin, the active principle of garlic. *Biochem. J.* **63,** 514 (1956).
2. Huh, K., Lee, S.I., Park J.M. and Kim, S.H.: Effect of diallyl disulfide on the hepatic glutathione S-transferase activity in rat. *Arch. Pharm. Res.* **9,** 205 (1986).
3. Boyland, E. and Chasseaud, L.F.: The role of glutathione and glutathione S-transferase in mercapturic acid biosynthesis. *Adv. Enzymol.* **32,** 173 (1969).
4. Habig, W.H., Pabist, M.J. and Jakoby, W.B.: Glutathione S-transferase: The first step in mercapturic acid formation. *J. Biol. Chem.* **249,** 7130 (1974).
5. Peter, M., Dean, P. J, Kari, O. and Sten, O.: Formation of a glutathione conjugate in isolated rat liver and kidney cells. *Biochem. Biophys. Res. Commun.* **83,** 195 (1978).
6. Prohaska, J.R. and Ganther, H.E.: Glutathione peroxidase activity of glutathione S-transferase purified from rat liver. *Biochem. Biophys. Res. Commun.* **76,** 437 (1977).
7. Burk, R.F., Nishiki, K., Lawrence, R.A. and Chance, B.: Peroxide removal by selenium-dependent and selenium-independent glutathione peroxidases in hemoglobin-free perfused rat liver. *J. Biol. Chem.* **253,** 43 (1978).
8. Schultz, E.M., Cragoe, E.J., Jr., Bicking, J.B., Bolhofer, W.A. and Sprague, J.A.: α,β-unsaturated ketone derivatives of aryloxyacetic acids, a new class of diuretics. *J. Med. Pharm. Chem.* **5,** 660 (1962).
9. Klaassen, C.D. and Fitzgerald, T.J.: Metabolism and biliary excretion of ethacrynic acid. *J. Pharmacol. Exp. Ther.* **191,** 548 (1974).
10. Yamada, T. and Kaplowitz, N.: Binding of ethacrynic acid to hepatic glutathione S-transferases *in vivo* in the rat. *Biochem. Pharmacol.* **29,** 1205 (1980).
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265 (1950).
12. Ellman, G.L.: Tissue sulfhydryl group. *Arch. Biochem. Biophys.* **82,** 70 (1959).

13. Bidlack, W.R. and Tappel, A.L.: Damage to microsomal membrane by lipid peroxidation. *Lipids,* **8,** 177 (1973).
14. Placer, I.A., Cushman, L.L. and Johnson, B.C.: Estimation of product of lipid peroxidation (malondialdehyde) in biochemical systems. *Anal. Biochem.* **16,** 359 (1966).
15. Cavallito, C.J., Bailey, J.H. and Buck, J.S.: Antibacterial principle of Allium sativum. III. Its precusor and essential oil of garlic. *J. Am. Chem. Soc.* **67,** 1032 (1945).
16. Dipaolo, J.A. and Carruthers, C.: Effect of allicin from garlic on tumor growth. *Cancer Research,* **69,** 793 (1977).
17. Jain, R.C.: Onion and garlic in experimental atherosclerosis. *Lancet,* **1,** 1240 (1975).
18. Bordia, A., Vyas, A.K., Khabya, B.L., Rathore, A.S., Bhu, N. and Bedi, H.: Effect of essential oil of onion and garlic on experimental atherosclerosis in rabbits. *Atherosclerosis,* **26,** 379 (1977).
19. Jain, R.C. and Vyas, C.R.: Garlic in alloxan-induced diabetic rabbits. *Am. J. Clin. Nutri.* **28,** 684 (1975).
20. Mathew, P. T. and Augusti, K.T.: Studies on the effect of allicin on alloxan-diabetes. I. Hypoglycaemic action and enhancement of serum insulin effect and glycogen synthesis. *Indian J. Biochem. Biophys.* **10,** 209 (1973).
21. Huh, K., Choi, C.W., Cho, S.Y. and Kim, S.H.: Effect of Allium sativum L. on the hepatic xanthine oxidase activity in rat. *J. Resource Development, Yeungnam University,* **2,** 111 (1983).
22. Huh, K., Lee, S.I. and Park, J.M.: Effect of garlic on the hepatic xanthine oxidase activity in rats. *Korean Biochem. J.* **18,** 209 (1985).
23. Dodds, M.G. and Foord, R.D.: Enhancement by potent diuretics of renal tubular necrosis induced by cephaloridine. *Br. J. Pharmacol.* **40,** 227 (1970).
24. Zimmerman, H.L.: *Hepatoxicity,* Appleton-Century-Crofts, New York (1978).
25. Bridges, J.W. and Chasseaud, L.F.: In Progress in Drug Metabolism: Pharmacokinetics and metabolism of diuretics (Ings, R.M.J. and Stevens, L. A., eds.,), vol. 7, John Wiley & Sons, New York, p98 (1983).
26. Plaa, G.L. and Witschi, H.: Chemicals, drugs and lipid peroxidation. *Am. Rev. Toxicol. Pharmacol.* **16,** 125 (1976).